QUANTIFICATION OF VIRAL RNA CONCENTRATIONS IN COMMERCIALLY AVAILABLE KILLED VACCINES FOR IBDV, BY REAL TIME RT-PCR.

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Abstract

In this study we standardized a method for viral RNA extraction from commercially available killed and live vaccines against infectious bursal disease virus (IBDV). We also used a prototype strains (STC) of know titer to prepare killed vaccine in our laboratory. We used the same live virus to develop a standard curve . We were able to show the differences in RNA content between the different vaccines.

Introduction

Vaccines provide protection against a potential threatening pathogen by inducing an immune response against the pathogen. If the titer of the pathogen in the vaccine is not adequate the response might not be sufficient to protect the animal. The objective of this study was to develop a method to extract the agent (viral RNA) from different commercially available killed and live vaccines against IBDV. We developed two different methods for viral extraction and found that modified chloroform-Trizol method of extraction gives better viral RNA concentration yield and real-time RT-PCR results as compared with modified chloroform-RNeasy method of RNA extraction

Materials and Methods

Live and killed virus vaccines

Four commercial killed virus vaccines and four live virus vaccines were obtained form company A, B, C and D. We also prepared a prototype killed virus vaccine form the classical virus strain STC in our lab.

RNA extraction for live and killed virus vaccines

1ml of chloroform was mixed with 0.5 ml of vaccine suspensions and then incubated it for 10 min at room temperature. The mixture was then centrifuged at 4000 RPM for 15 minutes. The upper aqueous layer was taken for RNA extraction by Trizol and RNeasy methods following the manufacturer instructions. The RNA concentration was then measured by *nano drop* spectrophotometer.

Real time RT-PCR for the killed and live virus vaccines

The real-time RT-PCR was performed on 7500 RT-PCR system (Applied Biosystems) both for killed and live virus vaccines. One step RT-PCR sensimix kit obtained from Quantace was used for RT-PCR master mix. The 743b.p primer for conserved VP2 region of IBDV(1) was used. The primer concentration was adjusted to 25 uM. The reaction profile was as follow: 45oC for 30 minutes, 95 oC for 10minutes, 95 oC for 15 seconds, 60 oC for 32 seconds and 72 oC for 1 minute.(2)

Results and Discussion

In case of killed virus vaccine we obtained 123.11ng/ul of RNA concentration for Vaccine- 2 (vac-2) 77.43 , 58.32 and 52.77 ng/ul for vaccines 1(vac-1) , vaccines-3(vac-3 and vaccine -4 (vac-4) respectively. The prototype vaccine STC gave higher RNA concentration of 378.38 ng/ul due to the fact that we used higher concentration of viral RNA (107 EID50) for vaccine preparation. (Fig.1)

Where as in case of live virus vaccines we observed 1314.42 ng/ul ,1025.76 ,820.72 and 780.31 ng/ul of viral RNA concentrations for live virus vaccines C, B, A and vaccine D respectively. (Fig.1). The Fig.3 &4 represent the melting curve and amplification plot for chloroform-Trizol LS extracted viral RNA and Fig.5 & 6 represent the Chloroform-RNeasy extracted RNA from killed vaccines.

Form our data we can deduce that different manufacturer use different virus concentration for vaccine preparation. This may be the fact that different vaccines induces varied level of protection against IBDV infection in the field conditions. This varied level of protection may also be attributed to the type of strains used for the vaccine preparation.

Real-Time RT-PCR for Killed Vaccines			
	Sample I.D.	**ng/ul**	**C.T. Value**
1	IBDV Killed Vaccine (Vac-1)	77.43	27.38
2	IBDV Killed Vaccine (Vac-2)	123.11	30.44
3	IBDV Killed Vaccine (Vac-3)	58.32	25.48
4	IBDV Killed Vaccine (Vac-4)	52.77	30.17
5	STC killed virus (Vac-5)	378.38	34*
Real-Time RT-PCR for Live Vaccines			
i	Live Vaccine (A)	820.72	33
ii	Live Vaccine (B)	1025.76	34
iii	Live Vaccine (C)	1314.42	28
iv	Live Vaccine (D)	780.31	32
v	STC Live virus	991.4	21.1**

* 1:10 dilution of RNA

** Positive control

Fig.1 Viral RNA concentration and CT values for killed and live vaccines

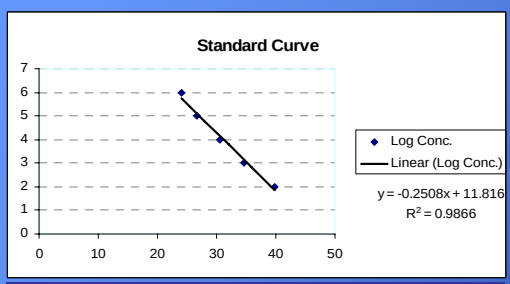

Fig.2 Standard Curve based on STC with SYBR® Green

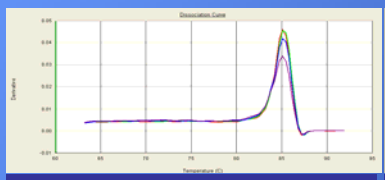

Fig.3 Melting Curve Analysis for killed vaccines with Chloroform-Trizol LS extracted RNA

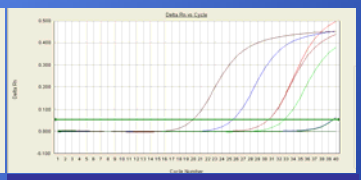

Fig.4 Real-Time RT-PCR Amplification plot for killed vaccines with Chloroform-Trizol LS extracted RNA

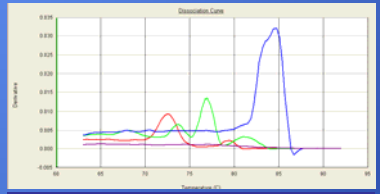

Fig.5 Melting Curve Analysis for killed vaccines with Chloroform-RNeasy extracted RNA

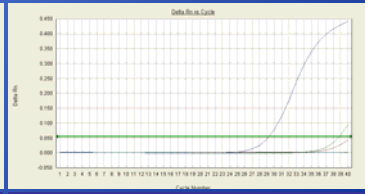

Fig.6 Real-Time RT-PCR Amplification plot for killed vaccines with Chloroform-RNeasy extracted RNA

References

1-Jackwood, D. J., and S. E. Sommer. 2002. Identification of infectious bursal disease virus quasispecies in commercial vaccines and field isolates of this double-stranded RNA virus. Virology 304:105-13.

2-Michelle A. Peters, T. L. L., Ching Ching Wu. 2005 Real-time RT-PCR differentiation and quantitation of infectious bursal disease virus strains using dual-labeled fluorescent probes. Journal of Virological Methods 127:87–95.